DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE OPPTS 850.1350

1. **CHEMICAL:** Dicamba acid — PC Code No.: 029801

2. **TEST MATERIAL:** Dicamba acid — Purity: 93.9%

3. **CITATION**

Authors:	Claude, M.B., T.Z. Kendall, and H.O. Krueger
Title:	Dicamba Acid: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Americamysis bahia*).
Study Completion Date:	February 13, 2012
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, MD 21601
Sponsor:	BASF Corporation 26 Davis Drive, P.O. Box 13528 Research Triangle Park, NC
Laboratory Report ID:	147A-277B
MRID No.:	48718012
DP Barcode:	402518

4. **REVIEWED BY:** David A. McEwen, Staff Scientist, CSS-Dynamac Corporation

Signature: David A. McEwen **Date:** 11/27/12

APPROVED BY: Mia Howard, Environmental Scientist, CDM Smith

Signature: Mia Howard **Date:** 01/25/13

5. **APPROVED BY:** Elizabeth Donovan, Biologist

Signature: Elizabeth Donovan Digitally signed by Elizabeth Donovan DN: cn=Elizabeth Donovan, o=EPA, ou=EFED, email=donovan.elizabeth@epa.gov, c=US Date: 2016.11.03 11:09:49 -04'00' **Date: 9/7/2016**

6. **STUDY PARAMETERS**

Age of Test Organism:	Neonates, <24 hours old
Definitive Test Duration:	35 days
Study Method:	Flow-through
Type of Concentrations:	Mean-measured

7. CONCLUSIONS:

Results Synopsis

NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Endpoint(s) Affected: None

8. ADEQUACY OF THE STUDY

A. Classification: ACCEPTABLE

B. Rationale: NA

C. Reparability: NA

9. MAJOR GUIDELINE DEVIATIONS:

1. The salinity of the dilution water should have been recorded at all levels. In this study, daily salinity measurements were performed in alternating replicates of the negative control group only.
2. The body lengths of mysids should have been determined at the time of sexual discernment (Day 14).
3. Survival of the offspring was not assessed.
4. The time to first brood release was not evaluated as a toxicological endpoint.

10. MATERIALS AND METHODS:

A. Biological System

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably *Americamysis bahia*.	Saltwater mysid, *Americamysis bahia*
Duration of the Test: 28 days	35 days [which was at least 7 days past the median time of the first brood release for the negative controls (Day 26)].
Source (or supplier) Should originate from in-house cultures.	In-house cultures
Parental Acclimation Within a 24-h period, changes in water temperature should not exceed 1°C, while salinity changes should not exceed 5% Mysids should be in good health.	Adult mysids were held in the laboratory for ≥14 days before juvenile collection. The culture was maintained in re-circulating laboratory saltwater (i.e., dilution water used for testing). During the 2-week period immediately preceding the test, the temperature ranged from 23.5 to 28.4°C, the pH ranged from 7.8 to 8.4, and the dissolved oxygen concentrations were ≥6.3 mg/L (≥85.9% saturation). The salinity of the filtered saltwater was 20 to 21‰. Mysids in the cultures were fed live brine shrimp nauplii (*Artemia* sp.) (Brine Shrimp Direct, Ogden, UT), which was periodically enriched with Algamac 3050 (Aquafauna Bio-Marine, Hawthorne, CA).

Guideline Criteria	Reported Information
Chamber Location: Treatments should be randomly assigned to test chamber locations.	Organisms were impartially distributed to the test compartments, and test compartments were indiscriminately assigned to exposure aquaria. The delivery system and test chambers were placed in a temperature-controlled environmental chamber.
Distribution: **No. of mysids before pairing**: Minimum of 40 mysids per concentration **No. of mysids after pairing**: Mysids should be separated into replicate groups of no more than eight individuals when most of the mysids reach sexual maturity (usually 10 to 14 days after test initiation).	60/level: 15 mysids per test compartment, 1 compartment per aquarium, and 4 replicate aquaria per treatment level. 20 pairs/level: 1 male:female pair per reproductive compartment, up to five reproductive compartments per replicate aquaria, and 4 replicate aquaria per treatment level.
Pairing: Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation)	Adult mysids were isolated and paired on Day 14 (aided by microscopic examination).
Offspring Exposure: Live young must be counted and separated into retention chambers at the same concentration where they originated.	Not conducted
Observations:	- Mortality, sub-lethal effects, and reproduction (post-pairing) were determined daily. - Offspring were observed for mortality and sub-lethal effects, and were counted and removed daily. - The total body lengths and dry weights of all surviving adult mysids were measured on Day 35.

Guideline Criteria	Reported Information
Feeding: Mysids should be fed during testing. A recommended food is live *Artemia* spp. Nauplii (*ca.* 48-hr old).	During the test, mysids were fed up to 4 times per day with live brine shrimp nauplii (*Artemia* sp.), that was periodically enriched with Algamac 3050. The food was also supplemented with the saltwater algae *Skeletonema costatum.* Concentrations and/or volumes offered per feeding were not reported.
Controls: Negative control and carrier control (when applicable) are required.	Negative and solvent control groups were included.

Comments: Following pairing, excess mature male organisms were maintained in a separate compartment within the replicate. If a male in a male/female pair died, it was replaced with a male, if available, from the pool of males maintained in the same replicate.

The in-life phase of the definitive test was conducted from October 25 to November 29, 2011.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural or artificial seawater 2) Natural seawater should be filtered (>20 µm) 3) Artificial seawater should be prepared with deionized (conductivity <0.1 mS/M at 12°C) or glass-distilled water. When deionized water is prepared from a natural water source, conductivity and TOC (or COD) should be measured in each batch.	Natural seawater collected at Indian River Inlet, Delaware was sand-filtered (25 µm), diluted to a salinity of *ca.* 20‰ with on-site well water, and aerated. Prior to use, the water was filtered again (0.45 µm) and UV-sterilized.
Salinity: 20 ± 3‰ (parts per thousand). Should be measured weekly in each chamber.	19 to 21‰ Measured daily in alternating replicates of the negative control group.
pH: Should be measured weekly in each chamber.	7.8 to 8.1 Measured at least weekly in alternating replicates from each level.
Dissolved oxygen: Should remain between 60 and 105% saturation. Should be measured weekly in each chamber.	≥77.7% saturation (≥5.7 mg/L) Measured in alternating replicates from each level at least weekly prior to pairing and daily following pairing.
Test Temperature: 25 ± 2°C Should be measured weekly in each chamber.	Weekly: 24.4 to 26.0°C Continuously: 24 to 26°C Measured in each test chamber at least weekly throughout the test, and continuously in one negative control replicate.

Guideline Criteria	Reported Information
Photoperiod: 14 hr light/10 hr dark with 15- to 30-minute transition periods	14 hr light/10 hr dark, with 120-minute transition periods. Light intensity was 198 lux over the surface of one representative test chamber at test initiation.
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) A dilution factor not greater than 0.5 and controls should be used.	1) Continuous-flow diluter 2) 5 toxicant concentrations 3) A dilution factor of 0.5 and appropriate controls were used.
Flow Rate: 1) Flow rates should provide ≥5 volume additions per 24 hr. 2) Flow splitting accuracy must be within 10%. 3) Meter systems calibrated before study and general operation checked twice daily during test period.	1) ≥18 volume additions/day for juvenile test chambers and ≥6 volume additions/day for adult test chambers. 2) Flow splitting accuracy was reported to be within ±10%. 3) The syringe pumps and rotameters were calibrated before the study, and the rotameters re-calibrated *ca.* weekly as needed throughout the study. The general operation of the diluter was checked visually once or twice per day.

Guideline Criteria	Reported Information
Test Vessels: 1) Materials and equipment that minimize sorption. 2) Should be loosely covered. **Retention Chambers:** Can be constructed with netting material of appropriate mesh size.	1) Prior to pairing: 9-L glass aquaria containing *ca.* 2.5 L of test solution (6.7 cm depth). After pairing: 19-L glass aquaria containing *ca.* 14.5 L of test solution (17.4 cm depth). 2) Not reported Prior to pairing: 2-L glass containers (12 cm diameter x 19 cm height) with nylon mesh screen attached to two holes on opposite sites (water depth of 6.3 cm). After pairing: 10 cm diameter glass Petri dishes with sides of nylon mesh screen (water depth of 16.6 cm).
Aeration: Permitted if necessary to maintain DO.	Gentle aeration was added to each test chamber beginning on Day 14.

Comments: During the 4-week period immediately preceding the study, the salinity of the dilution water was 20‰ and the pH ranged from 7.9 to 8.0 (n=4).

Results of periodic analysis of the dilution water for pesticides, organics, and metals were provided from water collected on 12/29/10.

Delivery of the test solutions into the test chambers was initiated 4 days prior to test initiation in order to achieve equilibrium of the test substance.

Chemical System:

Guideline Criteria	Reported Information
Test Item(s):	Identity: Dicamba acid Synonym: BAS 183 H(88) IUPAC name: 3,6-dichloro-o-anisic acid CAS name: 3,6-dichloro-2-methoxybenzoic acid CAS No.: 1918-00-9 Lot/Batch No.: 0002B01BA-251 Description: Solid Purity: 93.9% Storage: Ambient conditions
Concentrations: Concentration ranges should be selected to determine the concentration response curves, LC_{50} values, and MATC. Toxicant level should be measured at each level at 0, 7, 14, 21, and 28 days, and should not vary more than 20% among replicate test chambers.	Nominal: Solvent control, negative control, 0.75, 1.5, 3.0, 6.0, and 12 mg ai/L Mean-measured: <0.400 (<LOQ, solvent and negative controls), 0.69, 1.4, 2.9, 5.8, and 11 mg ai/L Water samples were collected from alternating replicate vessels (all levels) on Days 0, 7, 14, 21, 28, and 35. Additional samples were collected on Day 15 to confirm concentrations after gentle aeration was provided to each chamber. On Day 35, additional samples were collected and centrifuged due to the presence of a small amount of precipitate in the 12 mg ai/L group. Differences in centrifuged versus un-centrifuged sample results were negligible, and these results were not used to calculate mean-measured concentration. Analytical variation was minimal (<11% among replicates) (see copy of Excel worksheet in Appendix II).

Guideline Criteria	Reported Information
Solvents: 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, and ethanol.	Dimethylformamide (DMF, 0.02 mL/L)

Comments: Nominal concentrations were selected in consultation with the Sponsor, and were based upon exploratory range-finding data (not further specified).

During the definitive study, individual stock solutions were prepared for each level four times during the test. All concentrations were adjusted for the purity of the test item. A primary stock solution was prepared by mixing a calculated amount of the test material into HPLC-grade dimethylformamide (DMF) at a nominal level of 600 mg ai/mL. Four secondary stock solutions were then prepared in DMF at nominal concentrations of 37.5, 75, 150, and 300 mg ai/mL by proportional dilution of the primary stock. The stock solutions were mixed by inversion, and ranged in appearance from very light brown and clear to brown and clear, increasing in color intensity with an increase in concentration. Stock solutions were stored refrigerated in glass amber bottles, and aliquots placed into the syringe pump at 1- to 7-day intervals.

Water samples (processed immediately following collection) were analyzed for dicamba acid using HPLC with UV detection (220 nm). The limit of quantification (LOQ) was 0.400 mg ai/L.

Matrix blanks and matrix fortification samples (QC samples) were also prepared at each sampling interval and analyzed with each sample set. Matrix blank samples indicated that no interferences were observed at or above the LOQ. Recoveries from matrix fortification samples (fortified at 0.750, 3.00, or 12.0 mg ai/L) averaged 97.6 ± 1.15% (CV=1.18%) of nominal concentrations. It was not reported if sample results were adjusted for procedural recoveries.

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was performed in compliance with GLP standards as published by the U.S. EPA (40 CFR Parts 160 and 792), OECD [ENV/MC/CHEM(98)17], and Japan MAFF (11 NohSan, Notification No. 6283, 1999), with the following exception: periodic analyses of water for potential contaminants were not performed in accordance with GLP standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.
Controls: 1) Survival of the first-generation controls (between pairing and test termination) must not be less than 70%.	1) The average survival (between pairing on Day 14 and study termination on Day 35) was 82.5% for the negative control group and 82.5% for the solvent control group.
2) At least 75% of the paired 1st generation females in the controls produced young.	2) 80.0 and 94.1% of the paired 1st generation females produced young in the negative and solvent control groups, respectively.
3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	3) The average number of young produced by the 1st generation females was 6.0 and 13.3 in the negative and solvent control groups, respectively.

Guideline Criteria	Reported Information
Data Endpoints: 1) The number of dead adult mysids on Days 7, 14, 21, and 28. Concentration-response curves, LC_{50} values, and associated 95% C.I. for each interval. 2) The number of male and female mysids at the time of sexual discernment (usu. 10 to 12 days). 3) Body length of male and female mysids at the time of sexual discernment and again on Day 28. 4) Time to fist brood release. 5) Cumulative young per female. 6) If available, mortality, number of each sex, and body lengths of each sex should be recorded for the offspring. 7) Any abnormal behavior or appearance	**Endpoints evaluated in this study included:** 1) Cumulative survival of first-generation mysids on Day 14 (prior to pairing) and Day 35 (following pairing; not gender specific). 2) Determined (upon pairing) on Day 14. 3) Total length and dry weight of surviving mysids (gender specific) on Day 35. 4) Not evaluated 5) Mean number of young produced per female per reproductive day. 6) Number of live and dead offspring was determined. 7) Any abnormal behavior or appearance.
Raw data included?	Yes, sufficient

Toxicity Observations:

Adult (F_0): Survival from study initiation to pairing on Day 14 averaged 88.3 and 90.0% for the negative and solvent control groups, respectively, compared to 91.7, 90, 90, 90, and 78.3% in the mean-measured 0.69, 1.4, 2.9, 5.8, and 11 mg ai/L treatment levels, respectively. Juvenile survival was significantly ($p \leq 0.05$) decreased in the 11 mg ai/L level compared to the pooled control (89.2%). The NOAEC and LOAEC values calculated by the study author for juvenile survival were 5.8 and 11 mg ai/L, respectively, based on mean-measured concentrations.

Surviving mysids in the control and treatment groups generally appeared normal prior to pairing. The few observations of lethargy and smaller in comparison to mysids in the control groups were infrequent, not dose-dependent, and were comparable to the controls; therefore, these findings were not considered to be treatment-related.

Adult survival following pairing (Days 15 to 35) averaged 82.5% for the each of the negative and solvent control groups, and 85.7, 80.0, 69.2, 77.5, and 77.3% for the mean-measured 0.69, 1.4, 2.9, 5.8, and 11 mg ai/L treatment levels, respectively. Adult survival was not significantly decreased at

any concentration compared to the pooled control (82.5%). The NOAEC and LOAEC values for adult survival were 11 and >11 mg ai/L, respectively.

Surviving mysids in the control and treatment groups generally appeared normal. The few observations of lethargy were infrequent and were not considered to be treatment-related.

Toxicant Conc. (mg ai/L)		% Juvenile Survival Days 0 to 14	% Adult Survival Days 15 to 35	Mean Day to First Brood Release ± SD[b]	Mean No. Young per Reproductive Day ± SD
Nominal	Measured				
Negative	<LOQ[a]	88.3	82.5	26.4 ± 3.1	0.283 ± 0.136
Solvent	<LOQ[a]	90.0	82.5	26.1 ± 3.8	0.710 ± 0.278
Pooled	<LOQ[a]	89.2	82.5	--	--
0.75	0.69	91.7	85.7	25.2 ± 2.0	0.287 ± 0.068
1.5	1.4	90.0	80.0	24.9 ± 5.2	0.342 ± 0.241
3.0	2.9	90.0	69.2	25.3 ± 2.9	0.517 ± 0.119
6.0	5.8	90.0	77.5	28.2 ± 3.5	0.283 ± 0.048
12	11	78.3*	77.3	28.5 ± 4.1	0.176 ± 0.099

[a] LOQ = 0.400 mg ai/L
[b] Reviewer-calculated from raw data (see copy of Excel worksheet in Appendix III).
* Statistically-significant compared to the pooled control at $p \leq 0.05$.

The day of first brood release was reported as Day 16; no further discussion was provided, and this parameter was not assessed as a toxicological endpoint. The reviewer-calculated mean times to first brood release (see copy of Excel worksheet in Appendix III) were 26.4, 26.1, 25.2, 24.9, 25.3, 28.2, and 28.5 Days for the negative and solvent controls and mean-measured 0.69, 1.4, 2.9, 5.8, and 11 mg ai/L treatment levels, respectively. Based upon visual assessment of the data (by the reviewer), there appeared to be a treatment-related delay in the time to first brood release at the 5.8 and 11 mg ai/L treatment levels.

Reproduction was assessed in the study as the mean number of young produced per reproductive day, which averaged 0.283 and 0.710 for the negative and solvent control groups, respectively, compared to 0.287, 0.342, 0.517, 0.283, and 0.176 for the mean-measured 0.69, 1.4, 2.9, 5.8, and 11

mg ai/L levels, respectively. The mean reproduction values for the solvent control group were higher than the laboratory historical control data; therefore, the treated groups were compared to the negative control group only. Dunnett's test indicated that there were no significant decreases in reproduction in any treatment group compared to the negative control. The NOAEC and LOAEC values for reproduction were 11 and >11 mg ai/L, respectively, using mean-measured concentrations.

The growth of first-generation mysids was assessed as (gender-specific) total lengths and dry weights of surviving animals (on Day 35). Mean lengths of the males were unaffected by treatment and ranged from 7.68 to 7.97 mm in all dose groups including controls. Mean lengths of the females were 8.31 and 8.41 mm in the negative and solvent control groups, respectively, compared to 8.14, 8.30, 8.10, 8.06, and 8.11 mm in the mean-measured 0.69, 1.4, 2.9, 5.8, and 11 mg ai/L levels, respectively. This endpoint was significantly decreased ($p \leq 0.05$) at 2.9 and 5.8 mg ai/L compared to the pooled control (8.36 mm). However, the study authors noted that the differences between these treated groups and the pooled control were less than 4%, were not clearly concentration-dependent (no significant effect at the highest concentration), and were not accompanied by significant differences in mean dry weight. It was further reported that this finding is not likely to be biologically significant and may not be treatment-related. Mean dry weights of the males and females were unaffected by treatment and ranged from 0.93 to 1.07 mg and 1.15 to 1.41 mg, respectively, in all dose groups including controls. The NOAEC and LOAEC values for growth were 11 and >11 mg ai/L, respectively, based on mean-measured concentrations.

Toxicant Conc. (mg ai/L)		Total Length, (mm ± SD), Day 35		Dry Weight (mg ± SD), Day 35	
Nominal	Measured	♂	♀	♂	♀
Negative	<LOQ[(a)]	7.90 ± 0.120	8.31 ± 0.160	1.07 ± 0.052	1.26 ± 0.076
Solvent	<LOQ[(a)]	7.97 ± 0.167	8.41 ± 0.138	0.97 ± 0.071	1.38 ± 0.162
Pooled	<LOQ[(a)]	7.94 ± 0.139	8.36 ± 0.148	1.02 ± 0.075	1.32 ± 0.133
0.75	0.69	7.95 ± 0.175	8.14 ± 0.135	0.98 ± 0.032	1.38 ± 0.163
1.5	1.4	7.68 ± 0.060	8.30 ± 0.119	0.93 ± 0.050	1.39 ± 0.119
3.0	2.9	7.93 ± 0.146	8.10 ± 0.142*	0.98 ± 0.126	1.15 ± 0.100
6.0	5.8	7.86 ± 0.304	8.06 ± 0.153*	1.02 ± 0.094	1.26 ± 0.135
12	11	7.74 ± 0.097	8.11 ± 0.300	1.04 ± 0.088	1.41 ± 0.086

[(a)] LOQ = 0.400 mg ai/L

* Statistically-significant compared to the controls at $p \leq 0.05$.

Offspring (F_1): Not assessed.

Statistical Results:

Statistical Method: Statistical analyses were performed on survival (Days 14 and 35), the number of live young per reproductive day, and terminal length and dry weight of each surviving first-generation mysid (gender-specific).

Survival data (discrete-variable data) were analyzed using Chi-square and Fisher's Exact Test to identify treatment groups that showed a statistically significant difference from the pooled control ($p \leq 0.05$). Reproductive and growth data (continuous-variable data) were checked for normality using the Shapiro-Wilk's test and for homogeneity of variance using Levene's test ($p=0.01$). The data for reproduction, male dry weight, female dry weight, and female total length passed both assumptions, and were subsequently analyzed using analysis of variance (ANOVA) and Dunnett's test to identify treatments that were significantly different from the pooled controls ($p \leq 0.05$). The data for male total length did not pass the assumption of homogeneity of variance. When this data failed, a log transformation was performed. The log transformation did not correct the failure.

Therefore, the non-parametric Kruskal-Wallis test was used to identify any significant differences between the treated and pooled control groups.

The NOAEC and LOAEC were based on significance data, using scientific judgment to determine if statistical differences were biologically meaningful. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. All analyses were performed using SAS and TOXSTAT software and results were provided in terms of mean-measured concentrations.

Endpoint(s) Affected: juvenile survival

Most sensitive endpoint(s): juvenile survival

Endpoint	Method	NOAEC mg ai/L	LOAEC mg ai/L	MATC mg ai/L
Juvenile survival (Days 0 to 14)	Chi-square/Fisher's Exact Test	5.8	11	8.0
Adult survival (Days 15 to 35)	Chi-square/Fisher's Exact Test	11	>11	--
Offspring/repro. day	ANOVA/Dunnett's test	11	>11	--
Total length	ANOVA/Dunnett's test	11	>11	--
Dry weight	ANOVA/Dunnett's test	11	>11	--

Comments: None

12. REVIEWER'S STATISTICAL RESULTS:

Statistical method: The reviewer statistically analyzed the endpoints for juvenile survival (Day 14), adult survival (day 35), offspring/reproductive day, length (males and females), dry weight (males and females), and day of first brood. All analyses were performed using Toxstat 3.5 statistical software and all toxicity values were reported in terms of mean measured exposure concentrations. Replicate data were analyzed. The negative and solvent control groups were compared for each parameter using t-tests. There were no significant differences between the two control groups and all further analyses were made by comparing the treatment levels to the negative control only. The data were evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Levene's

tests, respectively. All data met all assumptions of normality and homogeneity of variance and therefore were analyzed using ANOVA followed by Dunnett's tests.

Juvenile survival
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Adult survival
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Offspring/reproductive day
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Length males
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Length females
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Dry weight males
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Dry weight females
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Day to first brood
NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L

Endpoints effected: None

Comments: The reviewer did not detect any significant adverse effects compared to the negative control at any exposure level. The study author detected a significant reduction in juvenile survival at the highest test level, compared to the pooled control group. The reviewer's results are reported in the Conclusions section.

13. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1350: *Mysid Chronic Toxicity Test.*

American Society for Testing and Materials. 2008. ASTM Standard E 1191-03a: *Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids.*

The SAS Institute, Inc. 1999-2001. The SAS System for Windows. Version 8.2. Cary, NC.

West, Inc. and D.D. Gulley. 1996. TOXSTAT® Release 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

Juvenile survival

Title: Dicamba acid mysid juvenile survival (day 14) %
File: 8012js Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 0.8832 Calculated t value = -0.2897
GRP2 (Solvent cntl) Mean = 0.9023 Degrees of freedom = 6
Difference in means = -0.0190

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid mysid juvenile survival (day 14) %
File: 8012js Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.2284
W = 0.9312

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba acid mysid juvenile survival (day 14) %
File: 8012js Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0270	0.0054	0.9582
Within (Error)	18	0.1016	0.0056	
Total	23	0.1286		

(p-value = 0.4687)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid mysid juvenile survival (day 14) %
File: 8012js Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0536	0.0107	0.8447
Within (Error)	18	0.2284	0.0127	
Total	23	0.2820		

(p-value = 0.5358)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid mysid juvenile survival (day 14) %
File: 8012js Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	0.8832	0.8832		
2	0.69	0.9165	0.9165	-0.4174	
3	1.4	0.9000	0.9000	-0.2103	
4	2.9	0.9250	0.9250	-0.5242	
5	5.8	0.9000	0.9000	-0.2103	
6	11	0.7833	0.7833	1.2555	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid mysid juvenile survival (day 14) %
File: 8012js Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

	NUM OF	MIN SIG DIFF	% OF	DIFFERENCE

GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1	neg control	4			
2	0.69	4	0.1920	21.7	-0.0333
3	1.4	4	0.1920	21.7	-0.0168
4	2.9	4	0.1920	21.7	-0.0418
5	5.8	4	0.1920	21.7	-0.0168
6	11	4	0.1920	21.7	0.1000

Adult survival

Title: Dicamba acid mysid adult survival (day 35) %
File: 8012as Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 0.7582 Calculated t value = -0.7465
GRP2 (Solvent cntl) Mean = 0.8678 Degrees of freedom = 6
Difference in means = -0.1095

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid mysid adult survival (day 35) %
File: 8012as Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.3798
W = 0.9845

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba acid mysid adult survival (day 35) %
File: 8012as Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0418	0.0084	1.2421
Within (Error)	18	0.1210	0.0067	
Total	23	0.1628		

(p-value = 0.3307)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid mysid adult survival (day 35) %
File: 8012as Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0801	0.0160	0.7593
Within (Error)	18	0.3798	0.0211	
Total	23	0.4599		

(p-value = 0.5906)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid mysid adult survival (day 35) %
File: 8012as Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	0.7582	0.7582		
2	0.69	0.8673	0.8673	-1.0612	
3	1.4	0.7990	0.7990	-0.3967	
4	2.9	0.6730	0.6730	0.8300	
5	5.8	0.7605	0.7605	-0.0219	
6	11	0.7828	0.7828	-0.2385	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid mysid adult survival (day 35) %
File: 8012as Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.69	4	0.2475	32.6	-0.1090
3	1.4	4	0.2475	32.6	-0.0408
4	2.9	4	0.2475	32.6	0.0852
5	5.8	4	0.2475	32.6	-0.0023
6	11	4	0.2475	32.6	-0.0245

Offspring/reproductive day

Title: Dicamba acid mysid offspring/reproductive day
File: 8012r Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 0.2831 Calculated t value = -2.7529
GRP2 (Solvent cntl) Mean = 0.7096 Degrees of freedom = 6
Difference in means = -0.4265

2-sided t value (0.05, 6) = 2.4469** Significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid mysid offspring/reproductive day
File: 8012r Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.3229
W = 0.9308

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba acid mysid offspring/reproductive day
File: 8012r Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0461	0.0092	1.0765
Within (Error)	18	0.1540	0.0086	
Total	23	0.2001		

(p-value = 0.4061)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid mysid offspring/reproductive day
File: 8012r Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.2546	0.0509	2.8390
Within (Error)	18	0.3229	0.0179	
Total	23	0.5775		

(p-value = 0.0463)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid mysid offspring/reproductive day
File: 8012r Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05

1	neg control	0.2831	0.2831		
2	0.69	0.2866	0.2866	-0.0370	
3	1.4	0.3419	0.3419	-0.6217	
4	2.9	0.5166	0.5166	-2.4653	
5	5.8	0.2825	0.2825	0.0061	
6	11	0.1756	0.1756	1.1346	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid mysid offspring/reproductive day
File: 8012r Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.69	4	0.2282	80.6	-0.0035
3	1.4	4	0.2282	80.6	-0.0589
4	2.9	4	0.2282	80.6	-0.2335
5	5.8	4	0.2282	80.6	0.0006
6	11	4	0.2282	80.6	0.1075

Length males

Title: Dicamba acid myside total length males (mm)
File: 8012lm Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 7.9050 Calculated t value = -0.6291
GRP2 (Solvent cntl) Mean = 7.9700 Degrees of freedom = 6
Difference in means = -0.0650

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid myside total length males (mm)
File: 8012lm Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.5220
W = 0.9761

```
     Critical W  = 0.8840 (alpha = 0.01 , N = 24)
              W  = 0.9160 (alpha = 0.05 , N = 24)

------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Dicamba acid myside total length males (mm)
File:            8012lm            Transform:            NO TRANSFORMATION

                 Levene's Test for Homogeneity of Variance

                              ANOVA Table
------------------------------------------------------------------------------

   SOURCE             DF              SS              MS           F
------------------------------------------------------------------------------
   Between             5            0.1014          0.0203       6.4802

   Within (Error)     18            0.0564          0.0031
------------------------------------------------------------------------------
   Total              23            0.1578
------------------------------------------------------------------------------
                                                    (p-value = 0.0013)

   Critical F  = 4.2479  (alpha = 0.01, df = 5,18)
               = 2.7729  (alpha = 0.05, df = 5,18)

   Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.01)


Title:  Dicamba acid myside total length males (mm)
File:            8012lm            Transform:            NO TRANSFORMATION

                              ANOVA Table
------------------------------------------------------------------------------

   SOURCE             DF              SS              MS           F
------------------------------------------------------------------------------
   Between             5            0.2409          0.0482       1.6612

   Within (Error)     18            0.5219          0.0290
------------------------------------------------------------------------------
   Total              23            0.7628
------------------------------------------------------------------------------
                                                    (p-value = 0.1950)

   Critical F  = 4.2479  (alpha = 0.01, df = 5,18)
               = 2.7729  (alpha = 0.05, df = 5,18)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.05)
```

Title: Dicamba acid myside total length males (mm)
File: 8012lm Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	7.9050	7.9050		
2	0.69	7.9500	7.9500	-0.3737	
3	1.4	7.6825	7.6825	1.8479	
4	2.9	7.9300	7.9300	-0.2076	
5	5.8	7.8650	7.8650	0.3322	
6	11	7.7375	7.7375	1.3911	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid myside total length males (mm)
File: 8012lm Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.69	4	0.2902	3.7	-0.0450
3	1.4	4	0.2902	3.7	0.2225
4	2.9	4	0.2902	3.7	-0.0250
5	5.8	4	0.2902	3.7	0.0400
6	11	4	0.2902	3.7	0.1675

Length females

Title: Dicamba acid myside total length females (mm)
File: 8012lf Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 8.3150 Calculated t value = -0.9442
GRP2 (Solvent cntl) Mean = 8.4150 Degrees of freedom = 6
Difference in means = -0.1000

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid myside total length females (mm)
File: 80121f Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.5736
W = 0.9474

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba acid myside total length females (mm)
File: 80121f Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0538	0.0108	1.7755
Within (Error)	18	0.1090	0.0061	
Total	23	0.1628		

(p-value = 0.1688)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid myside total length females (mm)
File: 80121f Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.2338	0.0468	1.4672

Within (Error)	18	0.5737	0.0319
Total	23	0.8074	

(p-value = 0.2491)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid myside total length females (mm)
File: 80121f Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	8.3150	8.3150		
2	0.69	8.1375	8.1375	1.4061	
3	1.4	8.3025	8.3025	0.0990	
4	2.9	8.1050	8.1050	1.6636	
5	5.8	8.0650	8.0650	1.9805	
6	11	8.1100	8.1100	1.6240	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid myside total length females (mm)
File: 80121f Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.69	4	0.3042	3.7	0.1775
3	1.4	4	0.3042	3.7	0.0125
4	2.9	4	0.3042	3.7	0.2100
5	5.8	4	0.3042	3.7	0.2500
6	11	4	0.3042	3.7	0.2050

Dry weight males

Title: Dicamba acid mysid dry weight males (mg)
File: 8012wm Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 1.0650 Calculated t value = 2.0647
GRP2 (Solvent cntl) Mean = 0.9750 Degrees of freedom = 6
Difference in means = 0.0900

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid mysid dry weight males (mg)
File: 8012wm Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.1123
W = 0.9832

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba acid mysid dry weight males (mg)
File: 8012wm Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0124	0.0025	1.1763
Within (Error)	18	0.0379	0.0021	
Total	23	0.0503		

(p-value = 0.3590)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid mysid dry weight males (mg)
File: 8012wm Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0433	0.0087	1.3882
Within (Error)	18	0.1122	0.0062	
Total	23	0.1555		

(p-value = 0.2753)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid mysid dry weight males (mg)
File: 8012wm Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	1.0650	1.0650		
2	0.69	0.9825	0.9825	1.4774	
3	1.4	0.9350	0.9350	2.3281	
4	2.9	0.9825	0.9825	1.4774	
5	5.8	1.0150	1.0150	0.8954	
6	11	1.0400	1.0400	0.4477	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid mysid dry weight males (mg)
File: 8012wm Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			

2	0.69	4	0.1346	12.6	0.0825
3	1.4	4	0.1346	12.6	0.1300
4	2.9	4	0.1346	12.6	0.0825
5	5.8	4	0.1346	12.6	0.0500
6	11	4	0.1346	12.6	0.0250

Dry weight females

Title: Dicamba acid mysid dry weight females (mg)
File: 8012wf Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 1.2650 Calculated t value = -1.3320
GRP2 (Solvent cntl) Mean = 1.3825 Degrees of freedom = 6
Difference in means = -0.1175

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba acid mysid dry weight females (mg)
File: 8012wf Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.2417
W = 0.9528

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba acid mysid dry weight females (mg)
File: 8012wf Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0105	0.0021	0.4965
Within (Error)	18	0.0759	0.0042	
Total	23	0.0864		

(p-value = 0.7748)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid mysid dry weight females (mg)
File: 8012wf Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.2096	0.0419	3.1211
Within (Error)	18	0.2417	0.0134	
Total	23	0.4513		

(p-value = 0.0335)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid mysid dry weight females (mg)
File: 8012wf Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	1.2650	1.2650		
2	0.69	1.3775	1.3775	-1.3729	
3	1.4	1.3950	1.3950	-1.5865	
4	2.9	1.1525	1.1525	1.3729	
5	5.8	1.2575	1.2575	0.0915	
6	11	1.4150	1.4150	-1.8306	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid mysid dry weight females (mg)
File: 8012wf Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL

1	neg control	4			
2	0.69	4	0.1975	15.6	-0.1125
3	1.4	4	0.1975	15.6	-0.1300
4	2.9	4	0.1975	15.6	0.1125
5	5.8	4	0.1975	15.6	0.0075
6	11	4	0.1975	15.6	-0.1500

Day of first brood

```
 Title:  Dicamba acid mysid time to first brood (days)
 File:        8012b          Transform:         NO TRANSFORMATION

     t-Test of Solvent and Blank Controls         Ho: GRP1 Mean = GRP2 Mean
================================================================================
GRP1 (Solvent cntl) Mean =    27.5000     Calculated t value =     1.0486
GRP2 (Blank cntl) Mean   =    26.1500     Degrees of freedom =     6
Difference in means      =     1.3500
================================================================================
2-sided t value (0.05, 6) = 2.4469  No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074  No significant difference at alpha=0.01

   WARNING:  This procedure assumes normality and equal variances!

Title:  Dicamba acid mysid time to first brood (days)
File:             8012b             Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
--------------------------------------------------------------------------------

     D =  95.6750
     W =   0.9791

     Critical W  = 0.8840 (alpha = 0.01 , N = 24)
              W  = 0.9160 (alpha = 0.05 , N = 24)

--------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Dicamba acid mysid time to first brood (days)
File:             8012b             Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE            DF               SS               MS           F
--------------------------------------------------------------------------------
```

Between	5	5.1633	1.0327	0.4433
Within (Error)	18	41.9350	2.3297	
Total	23	47.0983		

(p-value = 0.8125)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba acid mysid time to first brood (days)
File: 8012b Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	41.0633	8.2127	1.5451
Within (Error)	18	95.6750	5.3153	
Total	23	136.7383		

(p-value = 0.2258)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba acid mysid time to first brood (days)
File: 8012b Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control>Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	neg control	27.5000	27.5000		
2	0.69	25.3000	25.3000	-1.3495	
3	1.4	24.5000	24.5000	-1.8402	
4	2.9	25.4000	25.4000	-1.2882	
5	5.8	27.6250	27.6250	0.0767	
6	11	27.7250	27.7250	0.1380	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba acid mysid time to first brood (days)
File: 8012b Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control>Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.69	4	3.9288	14.3	-2.2000
3	1.4	4	3.9288	14.3	-3.0000
4	2.9	4	3.9288	14.3	-2.1000
5	5.8	4	3.9288	14.3	0.1250
6	11	4	3.9288	14.3	0.2250

APPENDIX II: COPY OF REVIEWER'S ANALYTICAL VARIATION CALCULATIONS:

Nominal Concentration (mg/L)	Time (Day)	Mean Measured Concentration (mg ai/L)	
0.75	0	0.649	
	7	0.695	
	14	0.703	
	15	0.666	
	21	0.706	
	28	0.706	
	35	0.679	
		Mean	0.686
		Standard Deviation	0.022
		Coefficient of variation (%)	3.3
1.5	0	1.37	
	7	1.37	
	14	1.47	

	15	1.37	
	21	1.41	
	28	1.49	
	35	1.35	
		Mean	1.40
		Standard Deviation	0.06
		Coefficient of variation (%)	3.9
3	0	2.95	
	7	2.8	
	14	3.05	
	15	2.95	
	21	2.79	
	28	2.85	
	35	2.87	
		Mean	2.89
		Standard Deviation	0.09
		Coefficient of variation (%)	3.2
6	0	5.57	
	7	5.74	
	14	5.93	
	15	5.77	
	21	5.77	
	28	5.93	
	35	6.11	
		Mean	5.83
		Standard Deviation	0.17
		Coefficient of variation (%)	3.0
12	0	11.2	
	7	11.1	
	14	12.8	
	15	8.91	
	21	11.3	
	28	12.1	
	35	11.9	

Mean	11.33
Standard Deviation	1.23
Coefficient of variation (%)	10.8

APPENDIX III. COPY OF EXCEL WORKSHEET CALCULATING TIME TO FIRST BROOD RELEASE:

Mean-measured Concentration (mg ai/L)	Replicate	Compartment	Day to First Brood	**Replicate means (bolded)**
Negative Control	A	1	30	
		2		
		3		
		4		
		5		**30.0**
	B	1	25	
		2	29	
		3	30	
		4	25	
		5		**27.3**
	C	1	25	
		2	20	
		3	28	
		4	25	
		5	23	**24.2**
	D	1		
		2	28	
		3	29	
		4		
		5		**28.5**
			Overall Mean	26.4
			Standard Deviation	3.1
Solvent Control	A	1	26	
		2	26	
		3	26	
		4		
		5		**26.0**
	B	1	26	
		2	24	
		3	17	
		4	34	
		5	31	**26.4**
	C	1	28	

		2	27	
		3	27	
		4	22	
		5	22	**25.2**
	D	1	26	
		2	29	
		3		
		4	26	
		5		**27.0**
			Overall Mean	26.1
			Standard Deviation	3.8
0.69	A	1	26	
		2		
		3	24	
		4	26	
		5	21	**24.3**
	B	1	27	
		2	24	
		3	28	
		4	24	
		5		**25.8**
	C	1	26	
		2	27	
		3	25	
		4		
		5	28	**26.5**
	D	1	28	
		2	24	
		3	24	
		4	24	
		5	23	**24.6**
			Overall Mean	25.2
			Standard Deviation	2.0
1.4	A	1	28	
		2	24	
		3		

		4	33	
		5	18	**25.8**
	B	1		
		2		
		3	20	
		4	16	
		5	24	**20.0**
	C	1	25	
		2	24	
		3	30	
		4	24	
		5	26	**25.8**
	D	1	24	
		2	32	
		3	30	
		4	16	
		5	30	**26.4**
			Overall Mean	24.9
			Standard Deviation	5.2
2.9	A	1	26	
		2	25	
		3		
		4		
		5	30	**27.0**
	B	1	30	
		2	21	
		3	25	
		4	28	
		5	25	**25.8**
	C	1		
		2	25	
		3	25	
		4		
		5		**25.0**
	D	1	23	
		2	23	
		3	23	
		4	21	

		5	29	**23.8**
			Overall Mean	25.3
			Standard Deviation	2.9
5.8	A	1	26	
		2	21	
		3	28	
		4	30	
		5		**26.3**
	B	1	26	
		2		
		3		
		4	24	
		5		**25.0**
	C	1	33	
		2	34	
		3	30	
		4	29	
		5	30	**31.2**
	D	1	27	
		2	24	
		3	30	
		4	31	
		5		**28.0**
			Overall Mean	28.2
			Standard Deviation	3.5
11	A	1	29	
		2		
		3	28	
		4	22	
		5		**26.3**
	B	1	27	
		2	33	
		3	33	
		4	30	
		5		**30.8**
	C	1		

		2	25	
		3		
		4		
		5		**25.0**
	D	1	35	
		2	22	
		3		
		4	28	
		5	30	**28.8**
			Overall Mean	28.5
			Standard Deviation	4.1